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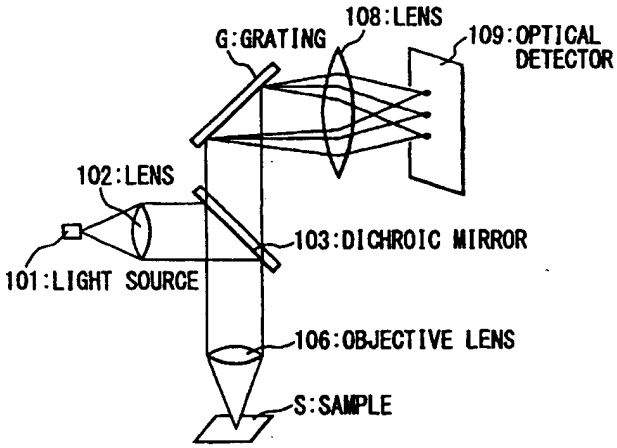
(54) Biochip reader and electrophoresis system

(57) The present invention provides a biochip reader, wherein light is irradiated at a biochip onto which multiple samples are arranged in spots or in linear arrays and image data according to the multiple samples is read using an optical detector. The biochip reader comprises means for arranging multiple pieces of spectroscopic information on the sample under analysis in spaces between images of the samples.

The present invention further provides a biochip reader comprising a light source for emitting excitation light, a dichroic mirror for reflecting or transmitting this light, an objective lens for condensing this light reflected or transmitted by the dichroic mirror and projecting fluorescent light produced at the biochip onto the dichroic mirror, an optical detector for detecting the fluorescent light, and a lens for condensing the fluorescent light reflected or transmitted by the dichroic mirror onto the optical detector.

The present invention further provides an electrophoresis system.

FIG. 6



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D scription

BACKGROUND OF THE INVENTION

Field f the Invention

[0001] The present invention relates to a reader for reading the wavelengths of fluorescence caused by marking such samples as deoxyribonucleic acid (DNA) or protein with a fluorescent substance and then exciting the substance by laser light or other alternative means. More specifically, the invention relates to improvements made in order to reduce the size and cost of the reader and increase the accuracy thereof.

[0002] The present invention further relates to a reader for biochips, such as DNA chips and protein chips. More specifically, the invention relates to a reader whose S/N ratio is superior and whose cost can be reduced.

[0003] The present invention still further relates to an electrophoresis system used in the field of bioengineering. More specifically, the invention relates to improvements made in order to increase the operating speed and resolution of the electrophoresis system.

D scription of the Prior Art

[0004] The prior art discloses a technique in which deoxyribonucleic acid (DNA) or protein is marked with a fluorescent substance, the substance is excited by irradiation with laser light, and the resulting wavelengths of fluorescence are read so that DNA or protein is detected and analyzed. In this technique, a biochip onto which samples of DNA or protein marked with the fluorescent substance are spotted in arrays is used.

[0005] The biochip is read by irradiating and scanning laser light laterally, for example, to excite spots of the fluorescent substance arranged in arrays. The emitted fluorescent light is then condensed by an optical fiber, for example, and received by an optical detector through an optical filter to detect the desired wavelength. When reading of one line (or array) of spots is completed, the biochip is moved longitudinally to repeat the same process as described above. This process is r peated until the biochip is read entirely.

[0006] Such a conventional biochip reader as discussed above has had the following problems, however.

1) The biochip has too many spots, is too large in terms of outside dimensions, and has too many arrays.

2) Fluorescence wavelengths are separated by means of an optical filter. It is therefore difficult to separate the wavelengths of polychrome fluorescent light since its spectra mix with each other depending on the concentration of each color.

3) The quantitativeness of measurement deteriorates due to th mixing of fluorescent light with self-emissions, background light or the like. This results in decreased accuracy.

4) A prolonged period of time is required wh n switching between optical filters and between optical detectors according to the fluorescence color.

5) Although the biochip reader can be speeded up by arranging multiple optical filters and optical detectors and letting the optical detector receive fluorescent light at the same time instead of switching between the filters and between the optical detectors, this approach has the problem of increased cost.

6) Using a scanning confocal microscope with the biochip reader involves an increase in the number of system components. This results in an increase in the system's cost and size, and also takes more time to perform measurement.

[0007] The object of the present invention is to solve the aforementioned problems by providing a biochip reader which can simultaneously achieve three objectives: downsizing, cost reduction and accuracy improvement.

[0008] A biochip, such as a DNA chip, used with the reader has the structure in which several thousand to several ten thousand types of known DNA segments are arranged in arrays on a substrate. If any unknown DNA segment is flowed onto the DNA chip, it combines with a DNA segment of the same type. Taking advantage of this nature of DNA, a known DNA segment that has formed a combination is examined by the biochip reader to identify the properties of the unknown DNA, such as DNA arrangement.

[0009] FIG. 1 shows an example of hybridizing such a biochip as described above. In FIG. 1, the six types of DNA segments, DN01 to DN06, are arranged in arrays on a substrate SB01 to form a DNA chip.

[0010] UN01 is an unknown DNA segment and is previously given a fluorescent mark, as indicated by LM01 in the figure. When hybridized to the DNA chip, this unknown DNA segment combines with another DNA segment whose arrangement is complementary.

[0011] For example, the unknown DNA segment UN01 combines with the known DNA segment DN01, as indicated by CB01 in FIG. 1.

[0012] Using the biochip reader, excitation light is irradiated at the DNA chip thus hybridized, in order to detect fluorescent light emitted from the fluorescent mark described earlier. Consequently, it is possible to know which of the known DNA segments the unknown DNA segment has combined with.

[0013] For example, in an image resulting from scanning the DNA chip indicated by SI01 in FIG. 1, fluorescent light is observed only at a spot where the DNA combination CB01 has been produced. This means fluorescent light is detected only from the spot indicated by LD01 in FIG. 1.

[0014] FIG. 2 is a schematic block diagram showing

an example of the conventional biochip reader described earlier. In FIG. 2, the numeral 1 indicates a light sourc for emitting excitation light, such as a laser light source, the numeral 2 indicates a dichroic mirror, the numeral 3 indicates an objective lens, the numeral 4 indicates a DNA chip which is a biochip onto which multiple cells are arranged in arrays, the numeral 5 indicates a filter, the numeral 6 indicates a lens, and the numeral 7 indicates an optical detector, such as a photomultiplier tube.

[0015] The symbols CL01 to CL03 are the aforementioned cells in which DNA segments, namely samples, of the same type are arranged.

[0016] Light emitted from the light source 1 is reflected by the dichroic mirror 2 as excitation light and condensed onto cells on the DNA chip 4 through the objective lens 3. For example, the excitation light is condensed onto the cell CL02.

[0017] Fluorescent light produced by the excitation light in the cell CL02 becomes parallel light as it travels through the objective lens 3, and passes through the dichroic mirror 2. Fluorescent light that has passed through the dichroic mirror 2 travels through the filter 5 and is condensed onto the optical detector 7 by the lens 6.

[0018] The DNA chip 4 is scanned by a drive means which is not shown in FIG. 2. For example, the DNA chip 4 is scanned in the direction indicated by MV01 in FIG. 2 so that the excitation light is irradiated at the remaining cells CL01 and CL03 on the DNA chip 4.

[0019] Consequently, it is possible to identify the arrangement of the unknown DNA segment from the position of a cell where fluorescence has taken place.

[0020] Dust may deposit on the DNA chip 4, however, for such reasons as the mixing of foreign matter with a liquid in which the unknown DNA segment is hybridized or the way subsequent processes are carried out. If the dust is organic, the excitation light causes the dust to emit fluorescent light that is more intense than that emitted by a cell. This results in the problem that the fluorescent light serves as noise and therefore deteriorates the S/N ratio.

[0021] FIG. 3 is an enlarged view of the cell CL02 shown in FIG. 2. Members indicated by 3, 4 and CL02 are the same as those in FIG. 2. If the DNA chip 4 is contaminated with dust particles marked DS01 and DS02 in FIG. 3, fluorescent light indicated by LL11 is produced by the excitation light in addition to fluorescent light emitted from the cell CL02. This deteriorates the S/N ratio.

[0022] For this reason, a confocal optical system has been used with the conventional biochip reader to detect only the fluorescent light produced by cells by removing fluorescent light produced by dust. Alternatively, a DNA chip has been h rmetically seal d to prevent it from being contaminated with dust. However, these measures have caused the problems not only of increased cost but also of insufficiently improved S/N ratio.

[0023] The objective of the present invention is therefore to provide a biochip reader whose S/N ratio is superior and whose cost can be reduced.

[0024] In addition, electrophoresis methods have been well known as means for analyzing the structure of genes or proteins, such as amino acid, using an inexpensive, simple system. The methods are very often used in the field of bioengineering. These electrophoresis methods include a disk electrophoresis method using polyacrylamide, an SDS (sodium dodecyl sulfate) polyacrylamide-gel electrophoresis method, an isoelectricpoint electrophoresis method, a nucleic-acid gel electrophoresis method, an electrophoresis method based on the effects of interaction with other molecules, a two-dimensional electrophoresis method, and a capillary electrophoresis method.

[0025] FIG. 4 shows an example of a conventional electrophoresis measurement system. The system consists mainly of two components, an electrophoresis unit 10 and a signal processor 20.

[0026] The electrophoresis unit 10 consists of a lane area 11, a first electrode 12 and a second electrode 13 for applying voltage to the lane area 11, a support plate 14 for supporting the lane area 11 and the first and second electrodes 12 and 13, a power unit 15 for electrophoresis used to supply voltage to the two electrodes, a light source 16 for emitting light to excite a fluorescent substance, an optical fiber 17 for guiding light emitted by the light source 16, and an optical detector 18 for condensing fluorescent light produced by a fluorescent substance to convert the light to an electric signal after selectively introducing light of a specific wavelength through an optical filter.

[0027] The signal processor 20 is designed to be able to receive an electric signal from the optical detector 18 to perform appropriate processes, such as converting the electric signal to digital data or performing preliminary processes, including summing and averaging. The output of the signal processor 20 is sent to a data processor, which is not shown in FIG. 4, where samples are submitted to an analysis process for examination.

[0028] In such a measurement system as described above, electrophoresis begins when a gel is injected into the lane area 11, samples of DNA segments marked with a fluorescent substance are injected from above the gel, and voltage is applied to the first and second electrodes 12 and 13 using the power unit 15. Molecules contained in the samples gather in each lane of samples as classified by molecular weight, each group of molecules forming a band. Since molecules with lower molecular weight have higher speeds of electrophoresis, they migrate longer distances within the same length of time.

[0029] These bands are detected by irradiating the gel with laser light, for example, emitted by the light source 16, causing marks of the fluorescent substance

that concentrate on the bands in the gel to emit fluorescent light, and detecting the fluorescent light with the optical detector 18.

[0030] That is, if the gel is irradiated with laser light, the fluorescent substance within part of the gel, which exists along a line L1 shown in FIG. 5, is excited to emit fluorescent light. This fluorescent light is detected at a given position of each lane, as it is searched for in the direction of electrophoresis with the lapse of time. Consequently, the fluorescent light is detected when a band B2 of each lane crosses the line L1. Thus, it is possible to obtain a signal representing the intensity pattern of fluorescence for a single lane.

[0031] The data processor, which is not shown in FIG. 5, is designed to be able to analyze each base sequence of DNA from the pattern signal.

[0032] Such a conventional electrophoresis system as described above has had the following problems, however.

[1] A prolonged period of time is required to perform measurement.

[2] The separability is not sufficient; too many lanes are required to separate a variety of DNA segments. Furthermore, information on the correlation among three or more dimensions is not available since the system is limited to two-dimensional analysis.

[3] The system requires a large installation space. For example, the lane area is as large as 50 cm × 50 cm or 5 cm × 5 cm.

[4] A two-dimensional system is particularly inferior in terms of positional reproducibility. This problem may be solved by applying markers to other lanes and then referencing them. However, applying markers in this way increases the lane area.

[0033] The object of the present invention is to solve the aforementioned problems by providing an electrophoresis system which has a compact lane area, offers highly accurate electrophoretic patterns, and permits faster acquisition of large amounts of interrelated information.

SUMMARY OF THE INVENTION

[0034] In order to achieve the aforementioned object, the present invention provides a biochip reader, wherein light is irradiated at a biochip onto which multiple samples are arranged in spots or in linear arrays and image data according to the multiple samples is read using an optical detector. The biochip reader comprises means for arranging multiple pieces of spectroscopic information on the sample under analysis in spaces between the images of the aforementioned samples. According to the biochip reader configured in such a way as described above, it is possible to output pieces of spectroscopic information on the samples into spaces between the images of the samples and thereby realize simultaneous, multi-wavelength measurement easily. According to this configuration, it is also possible to acquire multi-wavelength information using a compact biochip reader.

[0035] The present invention further provides a biochip reader comprising a light source for emitting excitation light, a dichroic mirror for reflecting or transmitting the excitation light, an objective lens for condensing the excitation light reflected or transmitted by the dichroic mirror and projecting fluorescent light produced at the biochip onto the dichroic mirror, an optical detector for detecting the fluorescent light, and a lens for condensing the excitation light reflected or transmitted by the dichroic mirror onto the detector. In this arrangement, the biochip is fabricated using a transparent substrate that can transmit both the excitation light and fluorescent light and the excitation light is irradiated from the side opposite to the side where the samples are arranged on the biochip. According to this configuration, it is possible to improve the S/N ratio of the biochip reader and reduce the cost thereof.

[0036] The present invention still further provides an electrophoresis system configured in such a manner that a sample marked with fluorescent coloring matter is allowed to migrate in a lane area and the pattern of fluorescence is read. The electrophoresis system comprises an electrophoresis unit for flowing multiple samples, which are prepared by combining a different type of fluorescent coloring matter with each of a variety of target substances, such as protein or DNA, through the same lane in the lane area, and a confocal scanner or a fluorescence imaging system which is configured in such a manner that the samples in the lane area are scanned with excitation light and the polychrome fluorescence patterns of samples that emitted fluorescent light by the irradiation of the excitation light are detected simultaneously through multiple filters with different transmission characteristics. According to this configuration, it is possible to reduce the number of lanes and thereby the size of the lane area, prevent the voltage gradient and the gel from becoming uneven, and perform precision measurement. Furthermore, it is possible to simultaneously detect polychrome fluorescence patterns using the confocal scanner or fluorescence imaging system and thereby reduce the time required for detection.

BRIEF DESCRIPTION OF THE DRAWINGS

[0037]

FIG. 1 is a schematic view showing an example of hybridization seen in biochips.

FIG. 2 is a schematic block diagram showing an example of a conventional biochip reader.

FIG. 3 is an enlarged view of a cell.

FIG. 4 is a schematic view showing an example of

a conventional electrophoresis system.

FIG. 5 is a schematic view showing a pattern of electrophoresis.

FIG. 6 is a schematic block diagram showing one embodiment of a biochip reader in accordance with the present invention.

FIG. 7 is a schematic view showing an arrangement of samples on a biochip.

FIG. 8 is a schematic view showing pieces of spectroscopic information indicated on an optical detector.

FIG. 9 is a schematic view showing pieces of spectroscopic information provided when samples arranged in linear arrays are measured.

FIG. 10 is a schematic block diagram showing another embodiment of the present invention.

FIG. 11 is a schematic view showing spectroscopic images obtained when pieces of spectroscopic information are developed in a two-dimensional way.

FIG. 12 is a schematic block diagram showing yet another embodiment of the present invention.

FIG. 13 is a schematic block diagram showing still another embodiment of the present invention.

FIG. 14 is a graph showing the distribution of self-emission, etc.

FIG. 15 is a schematic view showing the relationship between samples and apertures.

FIG. 16 is a schematic block diagram showing one embodiment of a biochip reader in accordance with the present invention.

FIG. 17 is a partially enlarged view of a cell when an immersion lens is used.

FIG. 18 is a partially enlarged view of a cell when a solid immersion lens (SIL) is used.

FIG. 19 is a schematic view showing comparison between DNA chips with and without an anti-reflection coating.

FIG. 20 is a schematic block diagram showing one embodiment of a polychrome electrophoresis system in accordance with the present invention.

FIG. 21 is a graph showing the distribution of wavelengths of excitation light and fluorescent light.

FIG. 22 is a schematic view showing the arrangement of samples and markers.

FIG. 23 is a schematic view showing a case where samples and markers are injected into the same lane.

FIG. 24 is a schematic view showing a lane area when threedimensional electrophoresis is conducted.

FIG. 25 is a schematic view showing a case where a lane on each axis is isolated.

FIG. 26 is a schematic view showing a case where markers are arranged along the depth of samples.

FIG. 27 is a schematic view showing the relationship between sample positions and apertur s.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0038] The present invention is described in detail below using the accompanying drawings. FIG. 6 is a schematic block diagram showing one embodiment of a biochip reader in accordance with the present invention.

[0039] In FIG. 6, the numeral 101 indicates a light source for emitting laser light, the numeral 102 indicates a lens for making parallel the laser light emitted by the light source 101, the numeral 103 indicates a dichroic mirror, the numeral 106 indicates an objective lens, the symbol S indicates a sample, the symbol G indicates a grating, the numeral 108 indicates a lens, and the numeral 109 indicates an optical detector.

[0040] Light (excitation light) emitted by the light source 101 is made parallel by the lens 102, is reflected by the dichroic mirror 103, is condensed through the objective lens 106, and is irradiated at the sample S. This irradiation causes the sample S to emit fluorescent light (whose wavelength differs from that of the excitation light). The fluorescent light then follows back the path that the excitation light followed, by passing through the objective lens 106 and reaching the dichroic mirror 103.

[0041] The fluorescent light that was emitted from the sample S and transmitted through the dichroic mirror 103 diffracts at the grating G. The diffraction angle of the fluorescent light is relative to its wavelength. The fluorescent light thus diffracted by the grating G is condensed onto the optical detector 109 through the lens 108. As the optical detector 109, a camera is used, for example.

[0042] If, for example, spots of four samples S1 to S4 are arranged on a biochip as shown in FIG. 7, spectroscopic images (spectra) with wavelengths of $\lambda 1$ to λn are formed for these respective samples in spatially different positions on the optical detector 109, as shown in FIG. 8. These spectroscopic images are spectroscopic information and can well be measured with a monochrome camera. As is evident from the figures, gaps between the spots are skillfully used in this example.

[0043] Although the embodiment described above is based on a biochip on which spots are placed sporadically in arrays, the present invention is not limited to this sample arrangement. The invention can also be applied to fluorescence patterns of electrophoresis arranged in linear arrays. In this case, images shown in FIG. 9 are obtained. That is, spectroscopic images with wavelengths of $\lambda 1$ to λn are formed for the electrophoresis pattern of each lane (along the longitudinal axis) in spatially different positions along the lateral axis.

[0044] FIG. 10 is a schematic block diagram showing another embodiment of the present invention. In the embodiment of FIG. 10, two gratings are arranged so that their directions of diffraction are at right angl s to each other. According to this configuration, two-dimensional spectra are obtained as shown in FIG. 11. If, for

example, the spectral pattern is graduated in 100-nm increments laterally (X-axis direction) and in 10-nm increments longitudinally (Y-axis direction), it is possible to perform measurement with a wider dynamic range and higher precision.

[0045] FIG. 12 shows an embodiment in which dichroic mirrors are used in place of the gratings. These dichroic mirrors are combinations of optical filters with optical shift means. As shown in FIG. 12, dichroic mirrors (optical filters) 31, 32 and 33 with different transmission wavelengths are stacked on the optical axis. In this embodiment, the angle of each dichroic mirror is determined so that light is reflected by the dichroic mirror at the same angle as it diffracts at a grating (equivalent to the optical shift means).

[0046] FIG. 13 is an embodiment in which a non-moving Fourier spectrometer 81, such as a Savart or a Michelson model, is used in place of the gratings or dichroic mirrors. In this embodiment, images formed at the optical detector 109 are not spectra themselves but an image of interference fringes. Consequently, spectra can be obtained by using computation means (not shown in the figure) and submitting this image to a Fourier transform process.

[0047] It should be noted that the measurement resolution can be further improved by using a confocal microscope or 2 photon microscope instead of a regular fluorescent substance or a camera. The quantitativeness of measurement is also improved because the slice effect of the confocal method makes it possible to always measure a constant volume of samples even if the thickness of each sample varies. In this embodiment, the confocal microscope may be of the non-scanning type.

[0048] As shown in FIG. 14, such noise as self-emissions whose wavelength slightly differs from that of the original fluorescent light can be removed easily because the properties of the reagent to be used are already known. If necessary, a signal spectrum may be separated using a regression method. With this approach, it is possible to easily achieve high precision and high sensitivity.

[0049] For spectroscopy, it is necessary to restrict the area of measurement using a shield means, such as slits. If the area of the shield means is greater than the area of a sample, dead spaces are produced in the imaging area of an optical detector. Conversely, if the area of the shield means is smaller than the area of the sample, dead spaces are produced in the area of the sample.

[0050] For this reason, an aperture A is optically aligned with the area of a sample S1 or with part of the sample S1, for example, as shown in FIG. 15 (A) and FIG. 15 (B). This arrangement makes it possible to most effectively use both the area of the sample S1 and the imaging area of the optical detector. This arrangement is also ffective for removing errors due to disorder in the edges of a sample. The shape of the apertur may not necessarily be circular; it may be rectangular instead.

[0051] The aperture shown in FIG. 15 (A) or FIG. 15 (B) or the rectangular aperture described above may be used as a pinhole or slit for a non-scanning confocal microscope. With this approach, it is possible for even a small and inexpensive microscope to achieve the high resolution characteristic of confocal microscopes and the quantitativeness due to the slice effect.

[0052] In this embodiment, the detection means is not limited to the spectroscopy method shown in FIG. 6, but may be a regular filter method.

Luminous energy can be increased further by attaching a microlens array MA to the light-source side of an aperture AP. Use of the microlens array MA eliminates the need for the aperture AP since light beams are condensed onto the focal point of each microlens.

[0053] As described above, the following advantages are offered in accordance with the present invention.

1) Multiple wavelengths of fluorescence can be measured simultaneously without having to change the filter and/or optical detector. It is therefore possible to realize a compact biochip reader.

2) A monochrome camera may be used to photograph spectra displayed on an optical detector for economical analysis.

3) Spectra displayed on an optical detector can be easily changed to two-dimensional spectra for higher precision.

4) The given area of a biochip can be most effectively used by aligning the aperture of excitation light or the spot of light condensed by a microlens array with a sample.

[0054] FIG. 16 is a schematic block diagram showing one embodiment of a biochip reader in accordance with the present invention.

[0055] In FIG. 16, members indicated by 1 to 3 and 5 to 7 are the same as those in FIG. 2, and the numeral 8 indicates a DNA chip using a plastic or glass substrate which allows excitation light or fluorescent light to pass through it. Members indicated by CL11 to CL13 are cells the same as those described earlier on which multiple samples of DNA segments of the same type are arranged. The symbols DS11 and DS12 indicate dust particles adhering to the cell CL12 on the DNA chip 8.

[0056] Light emitted as excitation light from a light source 1 is reflected by a dichroic mirror 2 and condensed onto a cell on the DNA chip 8 through an objective lens 3. At this point, the excitation light is irradiated from the side opposite to the side where the cells are arranged.

[0057] For example, the excitation light is irradiated at the cell CL12 through the transparent substrate of the DNA chip 8. Fluoresc nt light produc d by the excitation light at th cell is mad parallel through the objective

lens 3, and passes through the dichroic mirror 2. The fluorescent light that has passed through the dichroic mirror 2 is condensed by a lens 6 onto the optical detector 7 through a filter 5. At this point, the fluorescent light produced by the excitation light at the cell passes through the DNA chip 8 and is output to the side opposite to the side where the cells are arranged.

[0058] The DNA chip 8 is scanned by a drive means which is not shown in the figure. For example, the DNA chip 8 is scanned in the direction indicated by MV11 in FIG. 16 so that the excitation light is irradiated at the remaining cells CL11 and CL13 on the DNA chip 8.

[0059] Liquid in which unknown DNA segments are hybridized is flowed onto the side where the cells, such as the cell CL12, shown in FIG. 16 are arranged. The dust particles DS11 and DS12 adhere to the side of the substrate where the cells are arranged on the DNA chip 8.

[0060] On the other hand, no foreign matter such as the dust particle DS11 adheres to the side opposite to the side where the cells are arranged on the DNA chip 8.

[0061] Consequently, fluorescent light resulting from the dust particle and serving as a noise component can be reduced by irradiating the excitation light from the side opposite to the side where the cells are arranged on the DNA chip 8. For example, the excitation light is irradiated at the neighbors of a boundary between the substrate of the DNA chip and a cell.

[0062] In addition, a simple optical system can be used with the biochip reader and there is no need for hermetically shielding the DNA chip 8. These advantages make it possible to reduce the cost of the biochip reader.

[0063] It should be noted that although only a DNA chip is shown as an example of biochips when explaining figures, including FIG. 16, the biochips are, as the matter of course, not limited to a DNA chip only. They may be such chips as fabricated by arranging in arrays segments of ribonucleic acid (RNA), protein or sugar chain on a transparent substrate.

[0064] In this case, RNA segments undergo hybridization as with DNA segments, while protein segments and sugar chain segments are submitted to an antigen-antibody reaction. In either case, segments of known samples combine with segments of unknown samples marked with a fluorescent substance.

[0065] In addition, although the objective lens 3 shown in FIG. 16 is of the non-immersion type, it may be of the immersion type, such as a water immersion or an oil immersion lens. FIG. 17 is a partially enlarged view of the cell CL12 shown in FIG. 16 when an immersion lens is used. Members indicated by 3, 8 and CL12 in FIG. 17 are the same as those in FIG. 16.

[0066] In FIG. 17, the symbol LQ11 indicates a fluid such as water or oil filled into the gap between the objective lens 3 and the DNA chip 8. In this arrangement the numerical aperture (NA) is improved, thereby improving the S/N ratio further, because of the refractive index of fluid, such as water or oil. For this arrangement, however, the method in which beams of excitation light itself are scanned is more suitable than scanning the DNA chip 8 or the objective lens 3.

[0067] FIG. 18 is a partially enlarged view of the cell CL12 shown in FIG. 16 when a solid immersion lens (SIL), which has the same effect as an immersion lens, is used. In FIG. 18, members indicated by 8 and CL12 are the same as those in FIG. 16, and the numeral 9 indicates an SIL. Also, in this arrangement the numerical aperture (NA) is improved by the SIL, thereby improving the S/N ratio further.

[0068] If the substrate of a DNA chip 8 needs to be conductive, it may be prepared by placing transparent electrodes made of an indium-tin oxide (ITO) film on a transparent substrate. Hybridization can be accelerated by applying a positive voltage to the electrodes because DNA is charged with negative electricity.

[0069] An anti-reflection coating may be placed on the surface of the DNA chip 8's substrate opposite to the surface where cells are arranged.

[0070] FIG. 19 is a schematic view showing comparison between DNA chips with and without an anti-reflection coating.

[0071] In FIG. 19 (A), members indicated by 8 and CL12 are the same as those in FIG. 16, and the numeral 200 indicates an anti-reflection coating. The structure of the DNA chip 8 shown in FIG. 19 (A) is the same as the one shown in FIG. 16. In FIG. 19 (B), the anti-reflection coating 200 is formed on one side of the substrate of the DNA chip 8 opposite to the side where cells are arranged. In the case of FIG. 19 (A), the ratio of reflected light RL01 to incident light IL01 is approximately "4%". In the case of FIG. 19 (B), however, the ratio of reflected light RL11 to incident light IL11 can be reduced to as small as approximately "0.5%". Consequently, the luminous energy of excitation light irradiated at cells on the DNA chip 8 increases, improving the S/N ratio.

[0072] The side of the substrate of the DNA chip 8 where cells are arranged may be in the state of dryness. It is also possible to leave that side of the substrate wetted with hybridization liquid.

[0073] Although a laser light source has been mentioned earlier as an example of the source of excitation light, a non-laser light source, such as an LED lamp, xenon lamp, halogen lamp or any other white light source, may be used instead.

[0074] If a confocal optical system is used with the biochip reader, fluorescent light produced by dust particles can be removed more effectively. Consequently, it is possible to improve the S/N ratio further, compared with biochip readers with a non-confocal optical system.

[0075] As is evident from the explanations given above, the present invention offers the following advantages.

- The S/N ratio can be improved by irradiating excitation light from one side of the biochip opposite to the side where samples are arranged. Consequently, it is possible to reduce the cost of the biochip reader.
- The numerical aperture (NA) can be improved by using an immersion lens or a solid immersion lens (SIL) as the objective lens, thereby further improving th S/N ratio.
- The S/N ratio is still further improved, compared with biochip readers with a non-confocal optical system, because a confocal optical system is used with the biochip reader of the present invention.
- The luminous energy of excitation light irradiated at samples increases because the anti-reflection coating is formed on one side of the substrate of the DNA chip opposite to the side where the samples are arranged. Consequently, it is possible to further improve the S/N ratio.
- Transparent electrodes have been formed on a transparent substrate. This arrangement makes it possible to accelerate hybridization by applying a positive voltage to the electrodes because DNA is charged with negative electricity.
- If samples used with the biochip reader are either DNA or RNA segments, known samples having a complementary sequence combine by hybridization with unknown samples marked with a fluorescent substance. Consequently, it is possible to identify the sequence of the unknown samples.
- If samples used with the biochip reader are either protein segments or sugar chain segments, known samples combine by antigen-antibody reaction with unknown samples. Consequently, it is possible to identify the sequence of the unknown samples.

[0076] In several embodiments shown in FIG. 6 to FIG. 15, it is possible to use the types of samples mentioned above, that is, DNA, RNA, protein and sugar chain.

[0077] In several embodiments shown in FIG. 16 to FIG. 19, their optical detector may be one of the means shown in FIG. 6, FIG. 10, FIG. 12 and FIG. 13.

[0078] FIG. 20 is a schematic block diagram showing the features of one embodiment of a polychrome electrophoresis system in accordance with the present invention.

[0079] In FIG. 20, the numeral 100 indicates a confocal microscope and the numeral 300 indicates an electrophoresis unit.

[0080] The confocal microscope 100 (also referred to as the confocal optical scanner 100) is designed to be able to optically scan the gel in a lane area 201 and read the electrophoretic patterns of fluorescent light emitted from the gel. The confocal microscope 100 is configured as described below.

[0081] Excitation light (blue laser light with a wavelength of λ1, for example) emitted by a light source 101 is made parallel by a lens 102, is reflected by a dichroic mirror 103, and is condensed onto the slits of a slit array 105 through a lens 104. Excitation light that has passed through the slits is narrowed by an objective lens 106 and enters the gel in the lane area 201. The fluorescent substance in the lane area 201 is excited by this light and emits fluorescent light.

[0082] The fluorescent light thus produced retroactively follows the path that the xcitation light followed, by travelling through the objective lens 106 to the slit array 105 and the lens 104. It then passes through the dichroic mirror 103 to reach another dichroic mirror 107.

[0083] It should be noted that the dichroic mirror 103 reflects light with a wavelength of λ1 (blue, for example) and allows light with wavelengths greater than λ1 to pass through it. Likewise, the dichroic mirror 107 reflects light with a wavelength of λ2 (green, for example) and allows light with a wavelength of λ3 (red, for example) to pass through it. The relationship among λ1, λ2 and λ3 is shown in FIG. 21.

[0084] The light with a wavelength of λ2 that has been reflected by the dichroic mirror 107 is condensed onto an optical detector 109 through a lens 108. On the other hand, the light with a wavelength of λ3 that has passed through the dichroic mirror 107 is condensed onto an optical detector 111 through a lens 110.

[0085] If the slit array 105 is moved and controlled in such a manner that light emitted by the light source 101 scans across the surface of the lane area 201, the electrophoretic pattern of fluorescence produced in the lane area 201 is formed at each of the optical detectors 109 and 111. At this point, only the electrophoretic pattern of green fluorescence is formed at the optical detector 109, whereas only the electrophoretic pattern of red fluorescence is formed at the optical detector 111. The optical detectors 109 and 111 convert these images to electric signals and output them.

[0086] The electrophoresis unit 300 is equipped with the lane area 201 and power unit 202 for supplying voltage to cause electrophoresis in the lane area 201.

[0087] As described above, using a confocal optical scanner makes it possible to easily and precisely measure the polychrome electrophoretic pattern of fluorescence produced in the lane area 201.

[0088] It is not possible, however, to determine the absolute value of molecular weight by electrophoresis. Therefore, under normal conditions, reference marker molecules are flowed into neighboring lanes, as shown in FIG. 22. This method is problematic since it requires more space and involves measurement errors due to the difficulty in applying voltage evenly to all of the lanes.

[0089] In the present invention, a sample is flowed together with a reference marker molecule (hereinafter simply referred to as a "marker") into the same lane, as shown in FIG. 23. At this point, coloring matters with different fluorescence wavelengths ar combined with the respective markers and samples. A material thus pre-

pared is submitted to el ctrophoresis and scanned with the confocal optical scanner. Consequently, it is possible to detect two or more electrophoretic patterns of fluorescence at the same time.

[0090] FIG. 24 is another example of the embodiment of FIG. 20. Unlike the widely known two-dimensional electrophoresis, the embodiment of FIG. 24 is an example of three-dimensional electrophoresis in which another dimension is added in the direction of depth (Z-axis direction).

[0091] In this example, methods for applying a voltage gradient and a pH gradient in the X-axis (longitudinal), Y-axis (lateral) and Z-axis (depth) directions include:

1) applying high voltage in the X-axis direction, pH gradient in the Y-axis direction and low voltage in the Z-axis direction;
2) applying voltage in the X-axis direction, pH gradient in the Y-axis direction and multi-layer gel with each layer having a different concentration in the Z-axis direction; and
3) applying voltage in the X-axis direction, pH gradient in the Y-axis direction and a voltage gradient in the Z-axis direction, in order to perform affinity electrophoresis.

[0092] In this example, the electrophoresis system is configured so that the optically scanned surface of the lane area 201 can be moved up and down along the optical axis (in the Z-axis direction). For example, the electrophoresis system is configured so that the objective lens 106 of the confocal optical scanner 100 can be moved up and down. Then, X-Y axis polychrome electrophoretic patterns of fluorescence are detected by controlling the optically scanned surface in the Z-axis direction. Consequently, it is possible to easily acquire three-dimensional information.

[0093] In the explanation given above, only specific preferred embodiments are mentioned for the purpose of describing the present invention and showing examples of carrying out the invention. The above-mentioned embodiments are therefore to be considered as illustrative and not restrictive. The present invention may be embodied in other ways without departing from the spirit and essential characteristics thereof. Accordingly, it should be understood that all modifications falling within the spirit and scope of the present invention are covered by the appended claims.

[0094] For example, only the X-Z plane shown in FIG. 25 may be used as the lane in the embodiment of FIG. 24 to reduce the lane area, compared with that for two-dimensional electrophoresis. In addition, the distribution of concentration in the depth (Z-axis) direction can be realized by wetting only one side of the substrate with a highly concentrated solution or applying a density gradient in the depth direction by means of centrifugation. This distribution can also be realized by stacking multiple layers f gel with different concentrations.

[0095] If samples and markers are placed separately in the depth direction as shown in FIG. 26, it is possible to perform measurement using a compact electrophoresis system with all other conditions being the same as those of FIG. 5. In this case, the same fluorescence color may be used since lanes can be isolated in the depth direction by a confocal method.

[0096] When analyzing electrophoresis using a non-scanning confocal microscope, a sample may be positioned so that the aperture 61 of the confocal microscope is aligned with the sample position 62 or with part of the sample, as shown in FIG. 27. Consequently, it is possible to perform measurement with higher S/N ratios and without any adverse effect that may result when edges of the sample are measured.

[0097] As the light source, either a single-grating or 2 photon excitation light source may be used because they have the same effect.

[0098] As described above, the advantages offered by the present invention are as follows:

1) It is possible to easily realize highly precise polychrome electrophoresis using a compact system.
2) It is possible to realize three-dimensional electrophoresis using a compact system. In addition, large amounts of interrelated information can be acquired in a shorter length of time.

[0099] As also explained above, the three-dimensional electrophoresis system according to the present invention comprises:

1) an electrophoresis unit wherein various types of target substance, such as protein or DNA, are flowed into a lane area and the gradients of physical quantities such as voltage, pH, density and concentration, are used for electrophoresis; and
2) a scanning or non-scanning confocal microscope or 2 photon excitation microscope which is configured so that a sample in the lane area is scanned with excitation light and the fluorescence pattern of the sample produced by the excitation light is detected, whereby permitting detection of the three-dimensional position and the concentration of the sample.

[0100] In the electrophoresis system thus configured, one of the microscopes shown in the embodiments of FIG. 6 to FIG. 15 may be used in place of the scanning or non-scanning confocal microscope or the 2 photon excitation microscope mentioned above.

Claims

1. A biochip reader for reading image data according to a plurality of samples using an optical detector by irradiating light at a biochip whereupon said plural-

ity of samples are arranged in spots or arrays, comprising means for arranging multiple pieces of spectroscopic information of the sample under analysis in spaces among said images.

2. A biochip reader as defined in claim 1, wherein said means comprises a grating, a combination of an optical filter and optical shift means, or a Fourier spectrometer, arranged between said samples and said optical detector.

3. A biochip reader as defined in claim 1, wherein said means is configured so that spectroscopic information is developed on said optical detector in a two-dimensional manner if said samples are arranged in spots.

4. A biochip reader as defined in claim 1, wherein said means is a scanning confocal microscope, a non-scanning confocal microscope, or a dual-grating excitation microscope.

5. A biochip reader as defined in claim 1, comprising means for separating signals of said spectroscopic information from noise by using known spectra and a regression method.

6. A biochip reader as defined in claim 1, wherein an aperture for restricting the area of spectroscopy is aligned with the position of each sample or with part of each sample.

7. A biochip reader for reading image data according to a plurality of samples using an optical detector by irradiating light at a biochip whereupon said plurality of samples are arranged in spots or arrays, wherein reading means is a non-scanning confocal microscope having an aperture positioned to be optically conjugate with the position of the image of said sample or part of said sample in a given single image.

8. A biochip reader as defined in claim 7, wherein said non-scanning confocal microscope further comprises beam-condensing means on the light-source side of said aperture.

9. A biochip reader for reading image data according to a plurality of samples using an optical detector by irradiating light at a biochip whereupon said plurality of samples are arranged in spots or arrays, wherein reading means is a non-scanning confocal microscope having beamcondensing means, the focal point thereof being positioned to be optically conjugate with the position of the image of a sample or part of said sample in a given single image.

10. A biochip reader comprising:

a light source for emitting excitation light;

a dichroic mirror for reflecting said excitation or allowing said excitation light to pass through said dichroic mirror;

an objective lens for condensing light that has been reflected by or passed through said dichroic mirror onto a biochip and projecting fluorescent light produced at said biochip onto said dichroic mirror;

an optical detector for detecting said fluorescent light; and

a lens for condensing said fluorescent light that has been reflected by or passed through said dichroic mirror onto said optical detector,

wherein said biochip is configured using a transparent substrate allowing for passage of said excitation light and said fluorescent light and said excitation light is irradiated from one side of said biochip opposite to the side where samples are arranged.

11. A biochip reader as defined in claim 10, wherein said objective lens is an immersion lens.

12. A biochip reader as defined in claim 10, wherein said objective lens is a water immersion lens or an oil immersion lens.

13. A biochip reader as defined in claim 10, wherein said objective lens is an SIL.

14. A biochip reader as defined in claim 10, wherein an optical system is a confocal optical system.

15. A biochip reader as defined in claim 10, wherein an anti-reflection coating is formed on one side of said biochip opposite to the side where samples are arranged.

16. A biochip reader as defined in claim 10, wherein an anti-reflection coating is formed on a surface of said transparent substrate.

17. A biochip reader as defined in claim 16, wherein said anti-reflection coating is made of an indium-tin oxide film.

18. A biochip reader as defined in claim 10, wherein said samples are DNA segments.

19. A biochip reader as defined in claim 10, wherein said samples are RNA segments.

20. A biochip reader as defined in claim 10, wherein said samples are protein segments.

21. A biochip reader as defined in claim 10, wherein said samples ar sugar chain segments.

22. A biochip reader as defined in any of claims 1 to 9, wherein said biochip is configured using a transparent substrate allowing for passage of said excitation light and fluorescent light, and said excitation light is irradiated from one side of said biochip opposite to the side where samples are arranged.

23. An electrophoresis system for conducting electrophoresis of a sample marked with fluorescent coloring matter in a lane area so that a fluorescence pattern that is produced is read, comprising:

an electrophoresis unit for conducting electrophoresis by flowing a plurality of samples prepared by combining various types of target substance such as protein or DNA with different types of fluorescent coloring matter into the same lane of said lane area; and
a confocal microscope or a fluorescence imaging system wherein samples in said lane area are scanned with excitation light and polychrome fluorescence patterns of said samples produced by irradiating said excitation light are simultaneously detected through a plurality of filters having different transmission characteristics,
whereby a plurality of electrophoretic patterns are detected simultaneously.

24. A three-dimensional electrophoresis system for conducting electrophoresis of a sample marked with fluorescent coloring matter in a lane area so that a fluorescence pattern that is produced is read, comprising:

an electrophoresis unit for conducting electrophoresis by flowing various types of target substance such as protein or DNA into said lane area and applying a gradient of physical quantities such as voltage, pH, density or concentration in the direction of the depth of said sample; and
a scanning confocal microscope, a non-scanning confocal microscope, or 2 photon excitation microscope, which is configured so that a sample in said lane area is scanned with excitation light and a fluorescence pattern of said sample produced by irradiating said excitation light is detected,
whereby the three-dimensional position and concentration of said sample are detected.

25. A three-dimensional polychrome electrophoresis system as defined in claim 24, wherein different physical gradients are applied to said electrophoresis unit of said electrophoresis system in two horizontal directions and in one vertical direction so that sample separation is performed simultaneously on all three axes.

26. A three-dimensional electrophoresis system as defined in claim 24, wherein samples and markers are placed in the depth direction in said electrophoresis unit.

27. A three-dimensional electrophoresis system as defined in claim 24, wherein said non-scanning confocal microscope comprises an aperture positioned to be optically conjugate with the position of the image of said sample or part of said sample in a given single image.

28. A three-dimensional electrophoresis system as defined in claim 27, wherein said non-scanning confocal microscope further comprises beam-condensing means on the light-source side of said aperture.

29. A three-dimensional electrophoresis system as defined in claim 24, wherein said non-scanning confocal microscope comprises beam-condensing means, the focal point thereof being positioned to be optically conjugate with the position of the image of a sample or part of said sample in a given single image.

30. An electrophoresis system as defined in claim 23 or claim 24, wherein said confocal microscope has beam-condensing means on the light-source side of a confocal aperture.

31. An electrophoresis system as defined in claim 24, wherein the distribution of density in the depth direction is realized by wetting only one side of a gel with a highly concentrated solution, applying a density gradient in the depth direction by means of centrifugation, or stacking multiple layers of gel with different concentrations.

32. An electrophoresis system as defined in claim 24, wherein said scanning confocal microscope, non-scanning confocal microscope, or 2 photon excitation microscope, which is configured so that a sample in said lane area is scanned with excitation light and a fluorescence pattern of said sample produced by irradiating said excitation light is detected, is the biochip reader as defined in any of claims 1 to 3 or in any of claims 5 to 9.

FIG. 1 (PRIOR ART)

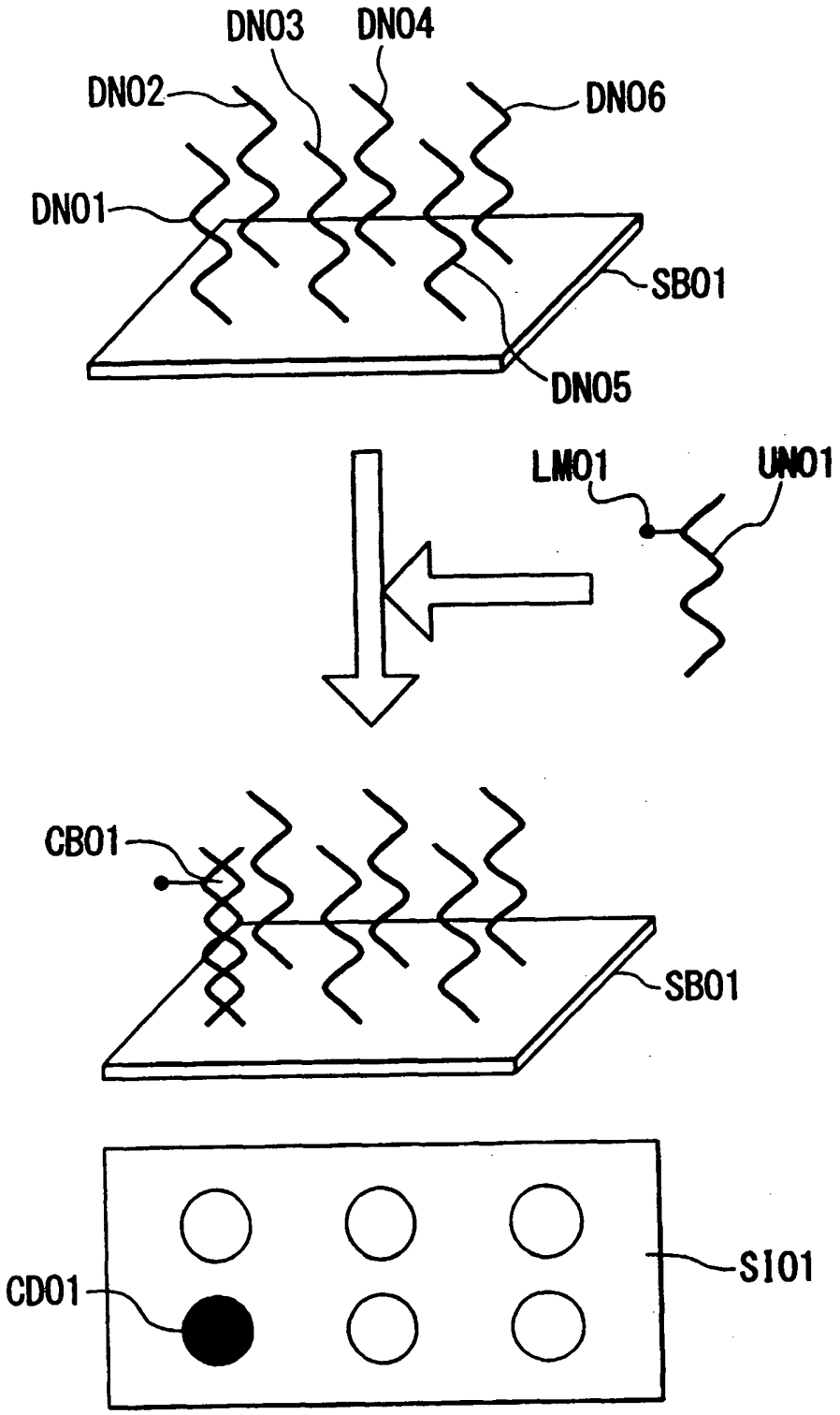


FIG. 2 (PRIOR ART)

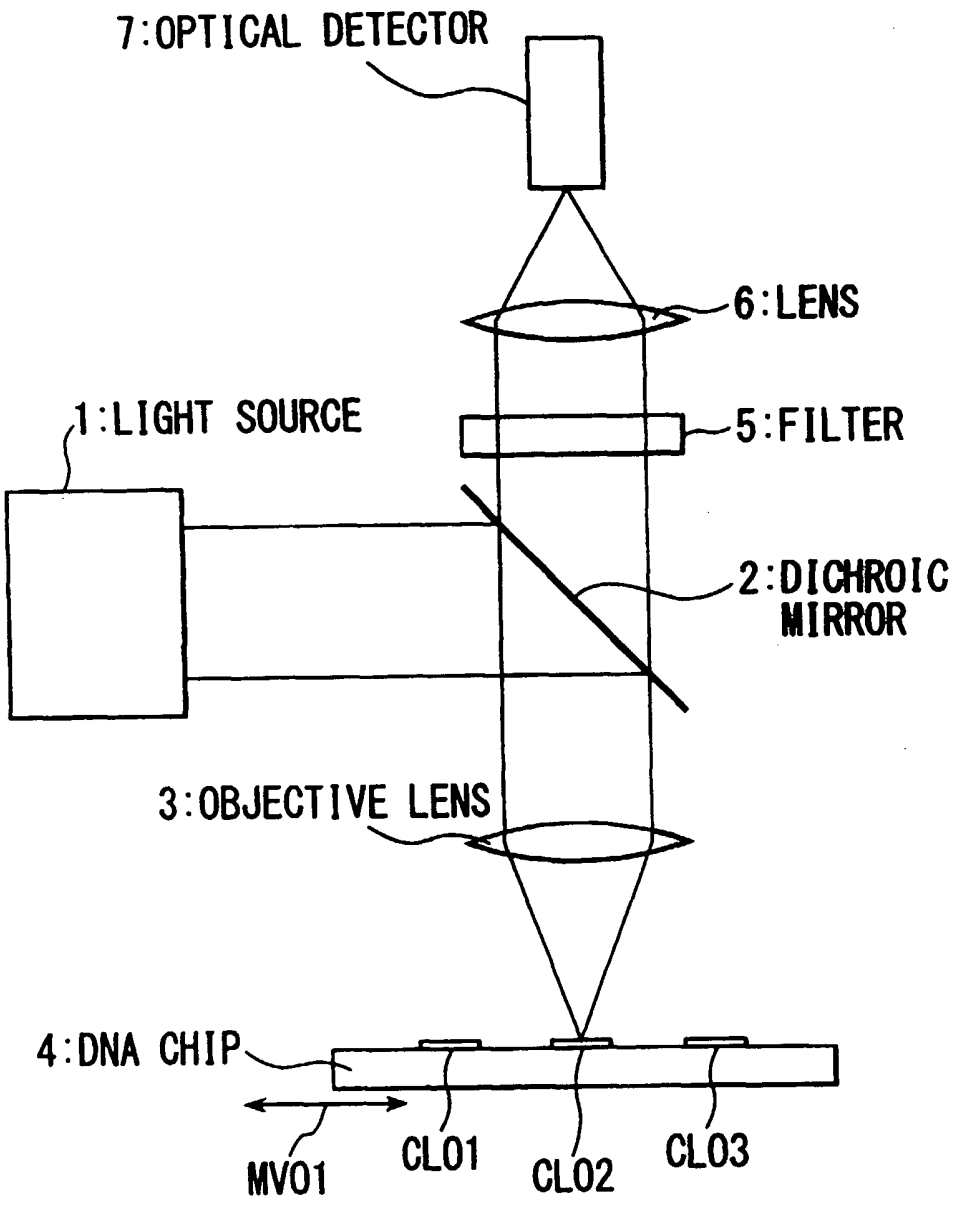


FIG. 3 (PRIOR ART)

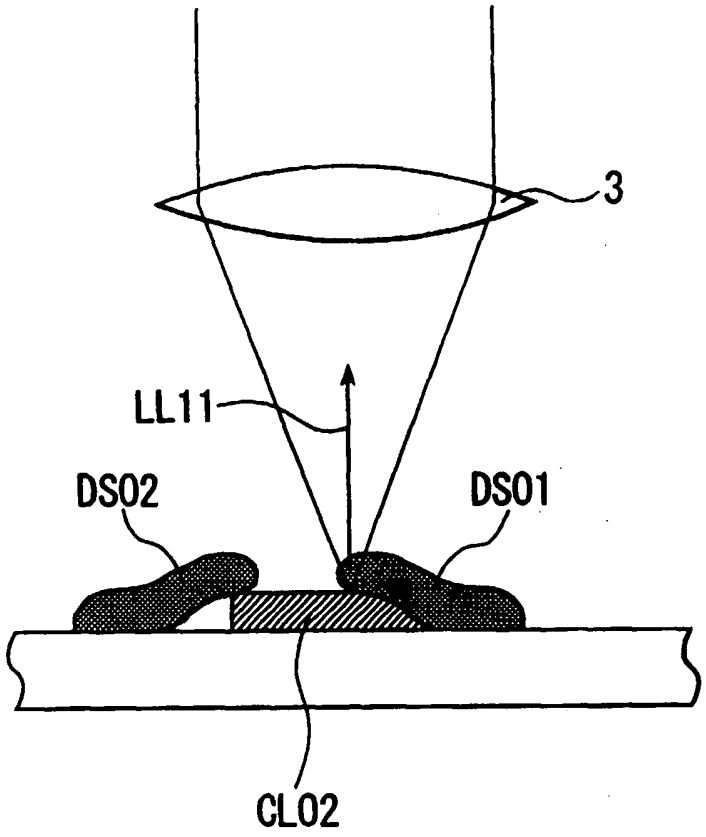


FIG. 4 (PRIOR ART)

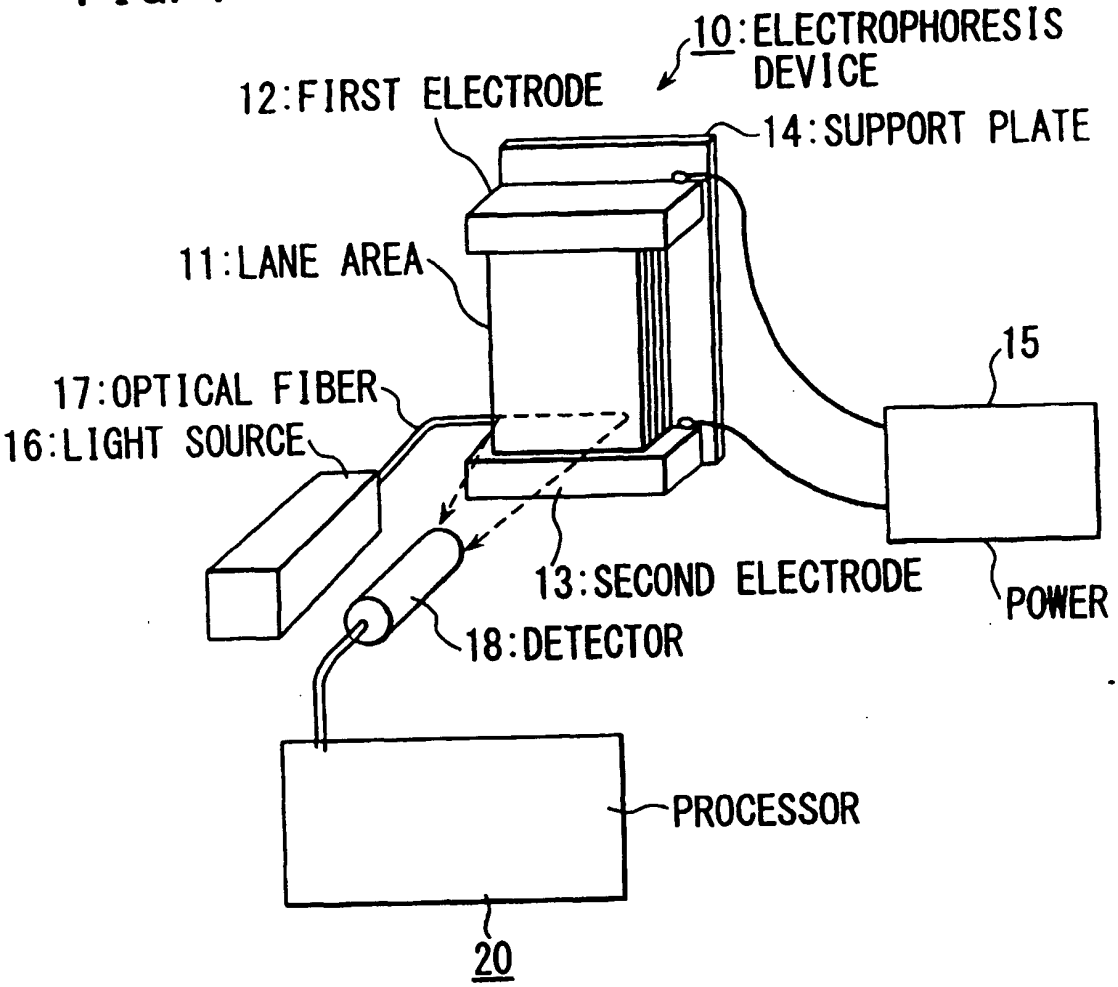


FIG. 5 (PRIOR ART)

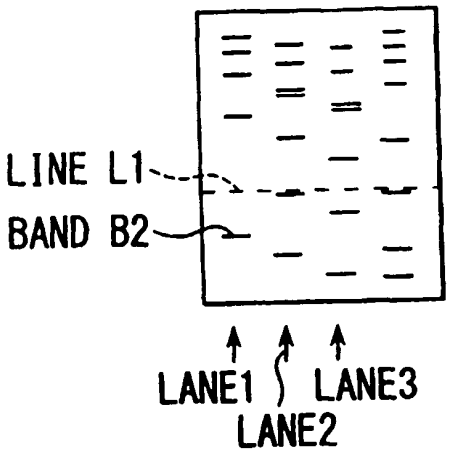


FIG. 6

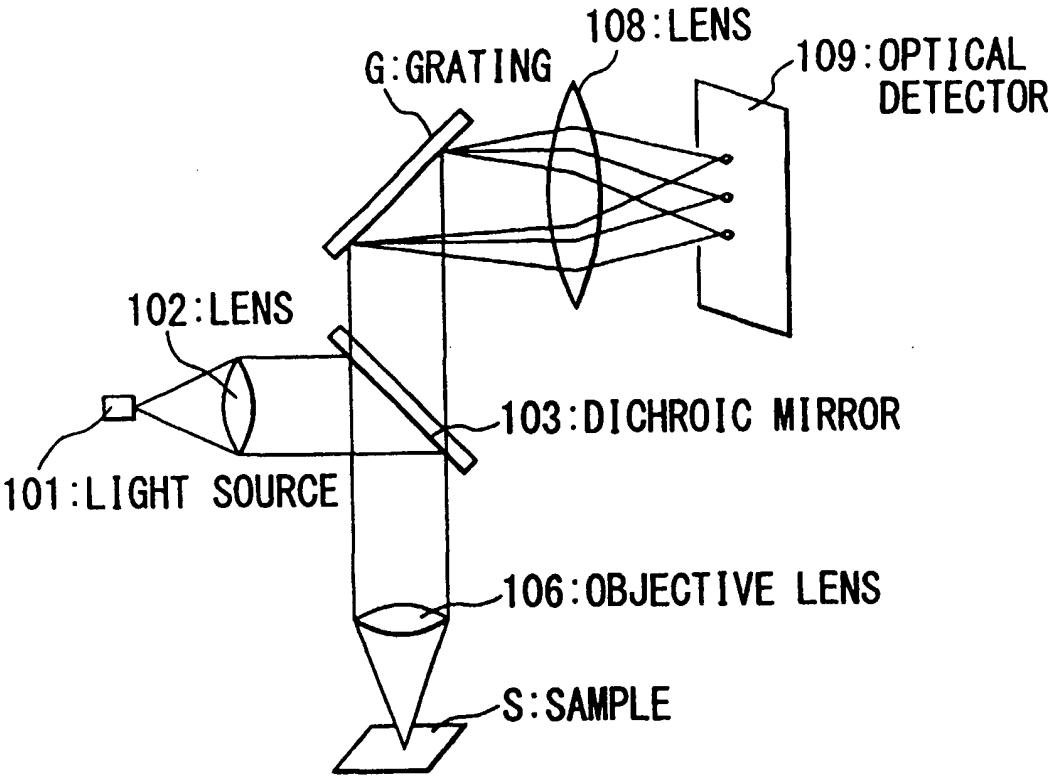

108:LENS
G:GRATING
109:OPTICAL DETECTOR
102:LENS
103:DICHROIC MIRROR
101:LIGHT SOURCE
106:OBJECTIVE LENS
S:SAMPLE

FIG. 7

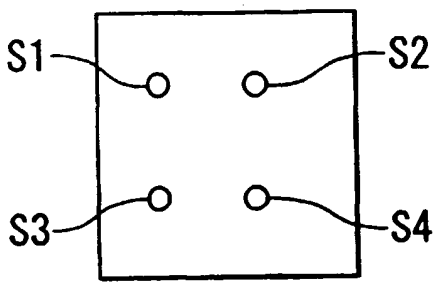

S1
S2
S3
S4

FIG. 8

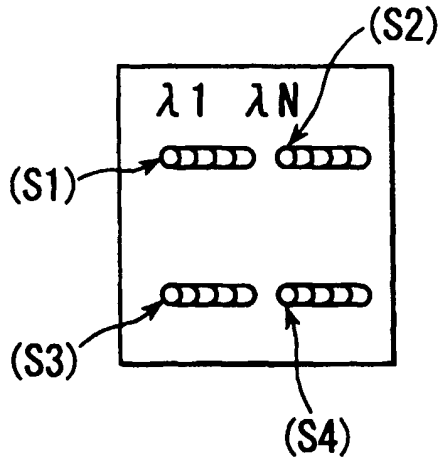


FIG. 9

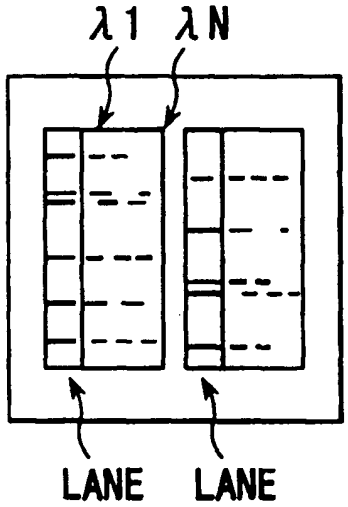


FIG. 10

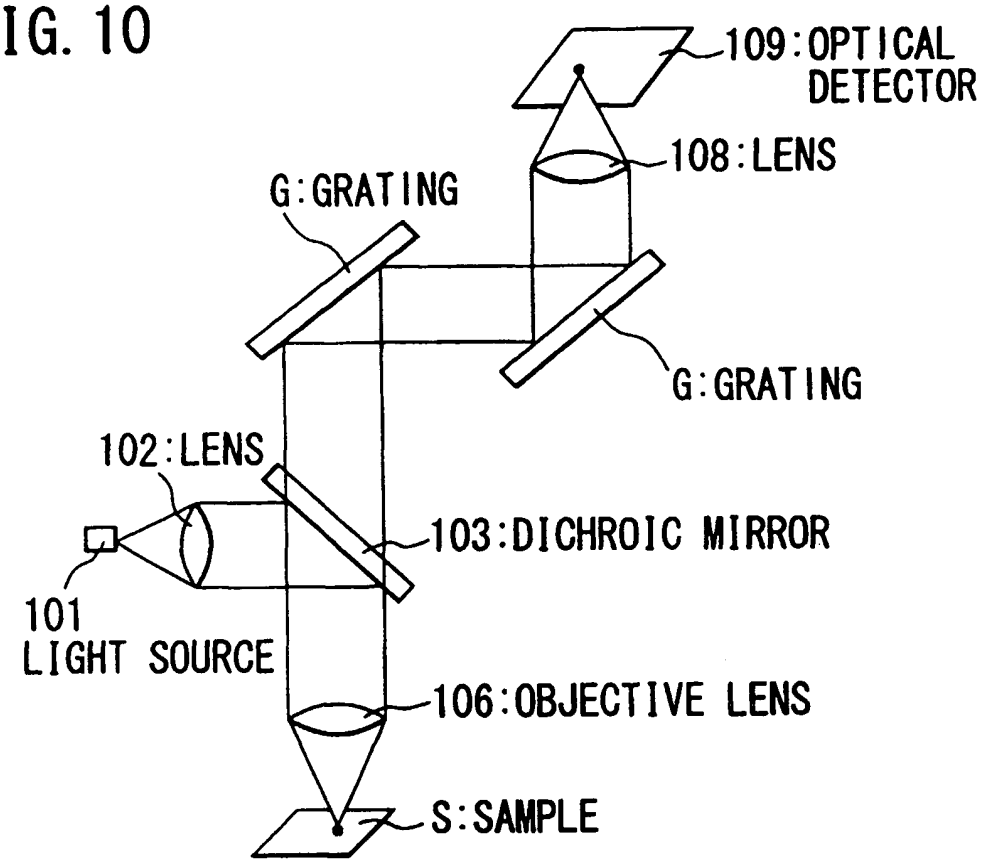

109:OPTICAL DETECTOR
108:LENS
G:GRATING
G:GRATING
102:LENS
103:DICHROIC MIRROR
101
LIGHT SOURCE
106:OBJECTIVE LENS
S:SAMPLE

FIG. 11

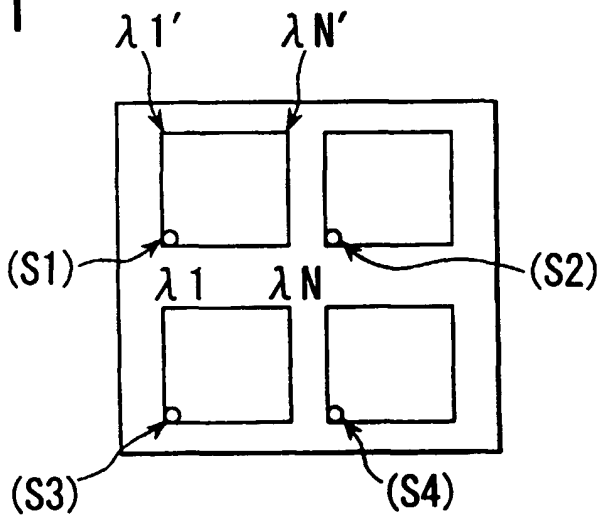

λ1'
λN'
(S1)
(S2)
λ1
λN
(S3)
(S4)

FIG. 12

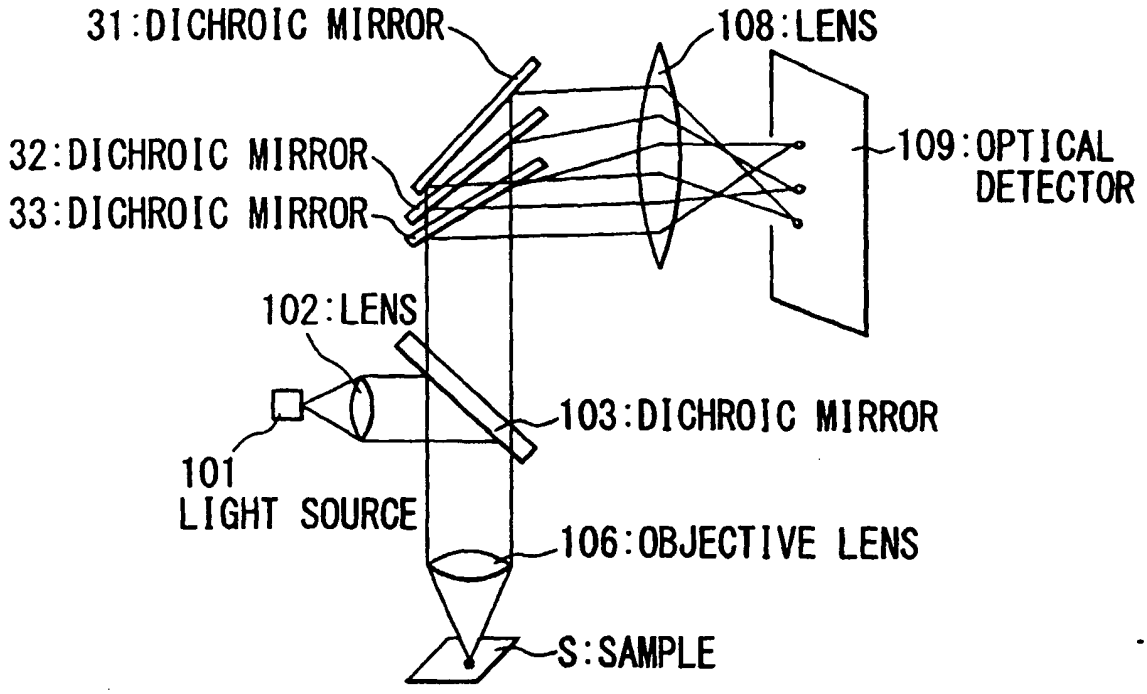

31:DICHROIC MIRROR
108:LENS
32:DICHROIC MIRROR
33:DICHROIC MIRROR
109:OPTICAL DETECTOR
102:LENS
103:DICHROIC MIRROR
101
LIGHT SOURCE
106:OBJECTIVE LENS
S:SAMPLE

FIG. 13

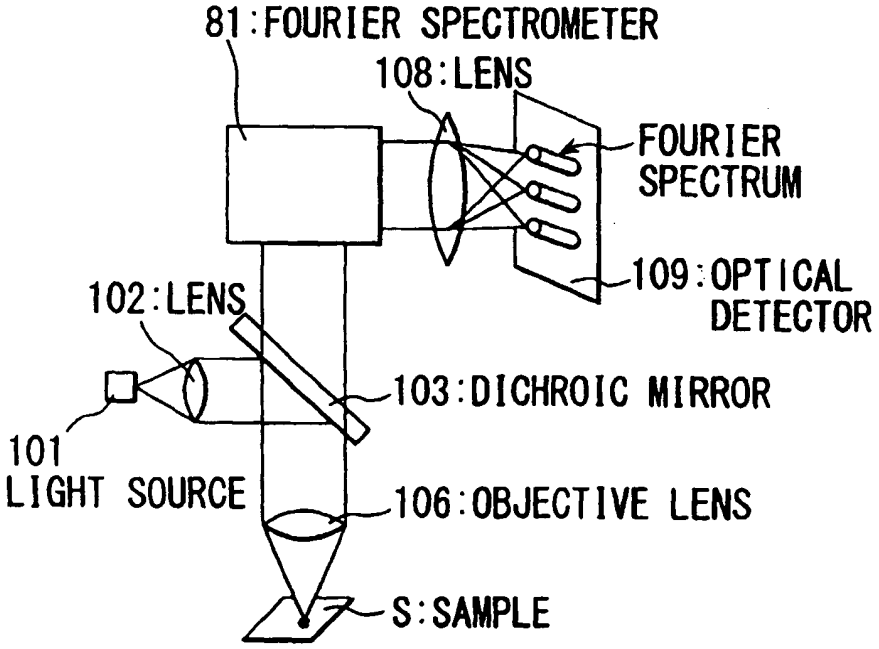

81:FOURIER SPECTROMETER
108:LENS
FOURIER SPECTRUM
109:OPTICAL DETECTOR
102:LENS
103:DICHROIC MIRROR
101
LIGHT SOURCE
106:OBJECTIVE LENS
S:SAMPLE

FIG. 14

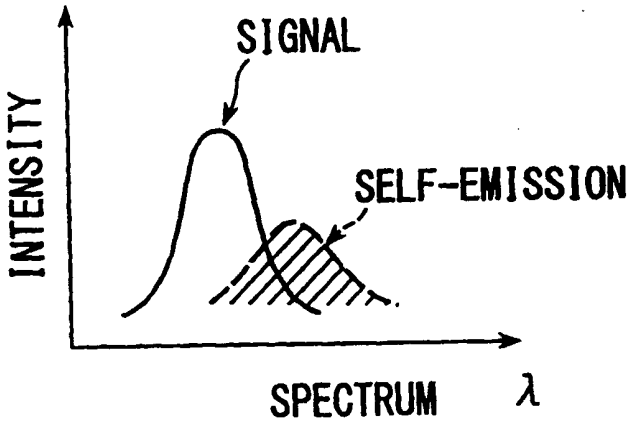
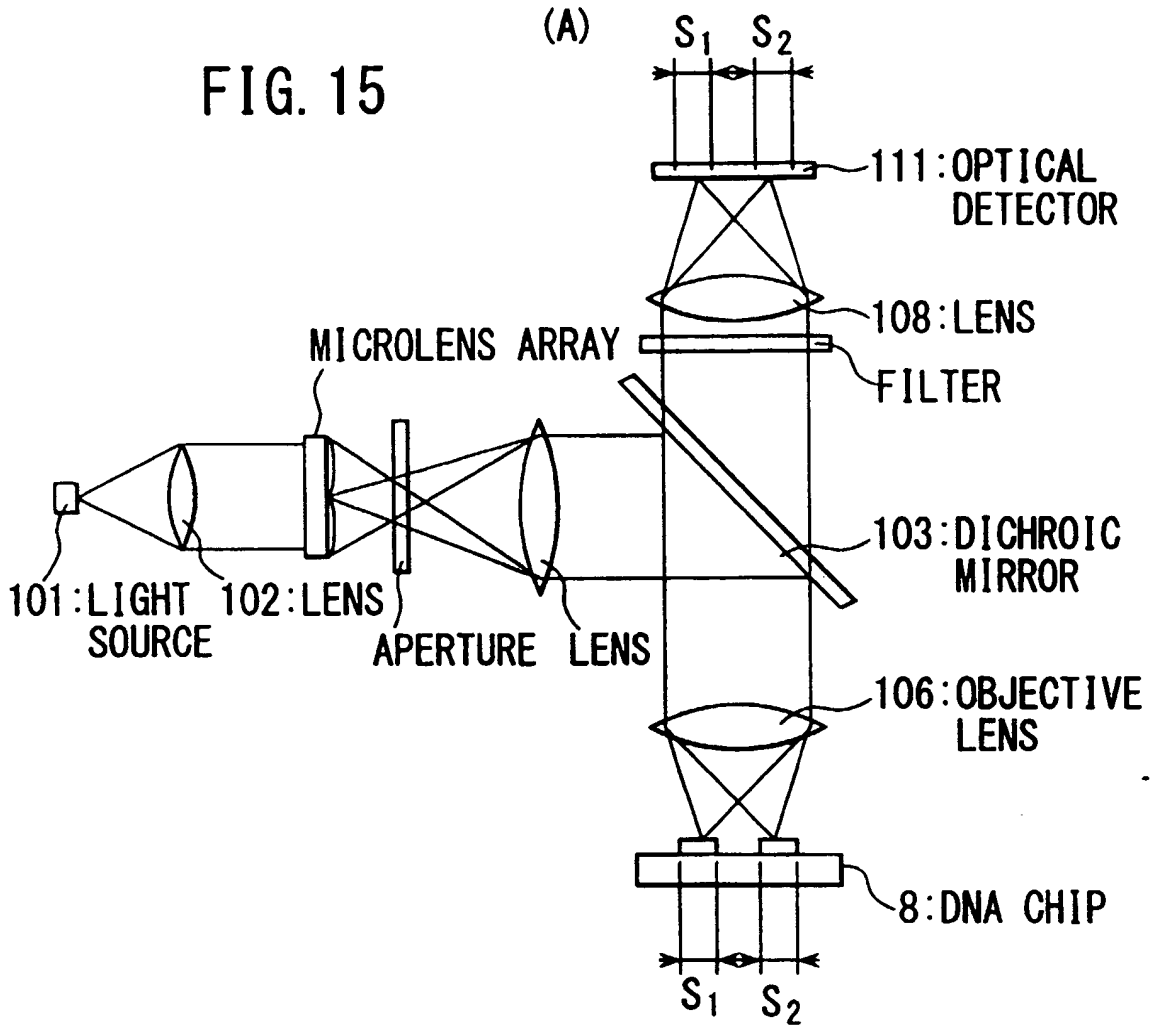


FIG. 15


(A)
S1 S2
111:OPTICAL DETECTOR
108:LENS
MICROLENS ARRAY
FILTER
103:DICHROIC MIRROR
101:LIGHT SOURCE
102:LENS
APERTURE
LENS
106:OBJECTIVE LENS
8:DNA CHIP
S1 S2

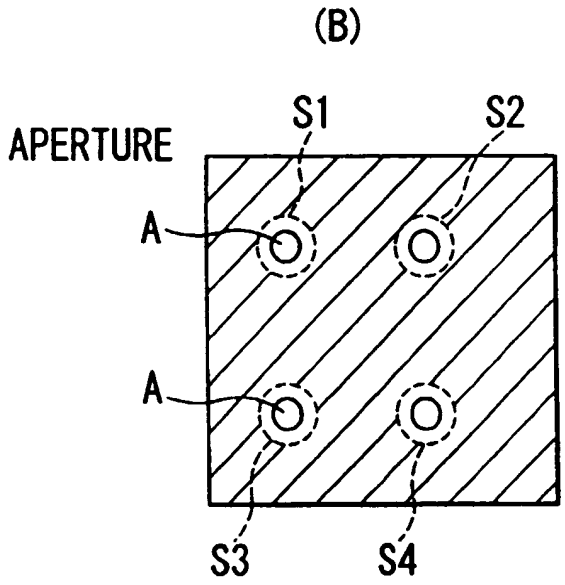

(B)
APERTURE
S1
S2
A
A
S3
S4

FIG. 16

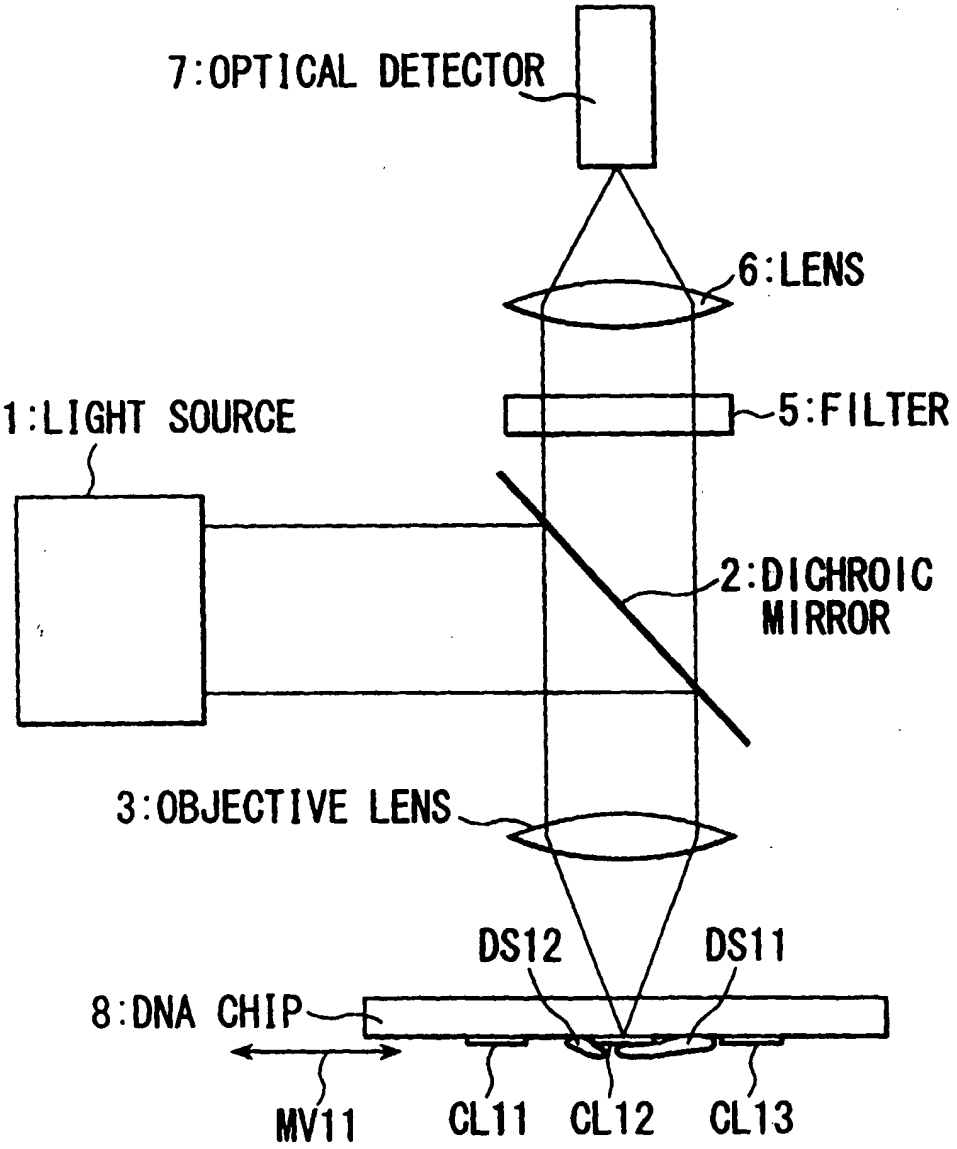

7:OPTICAL DETECTOR
6:LENS
1:LIGHT SOURCE
5:FILTER
2:DICHROIC MIRROR
3:OBJECTIVE LENS
DS12
DS11
8:DNA CHIP
MV11
CL11
CL12
CL13

FIG. 17

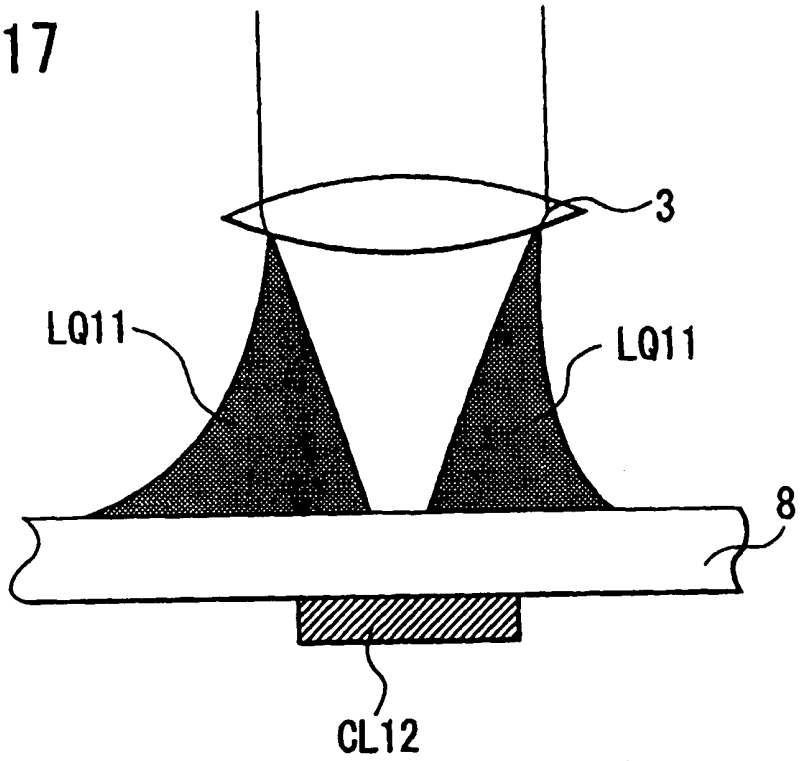


FIG. 18

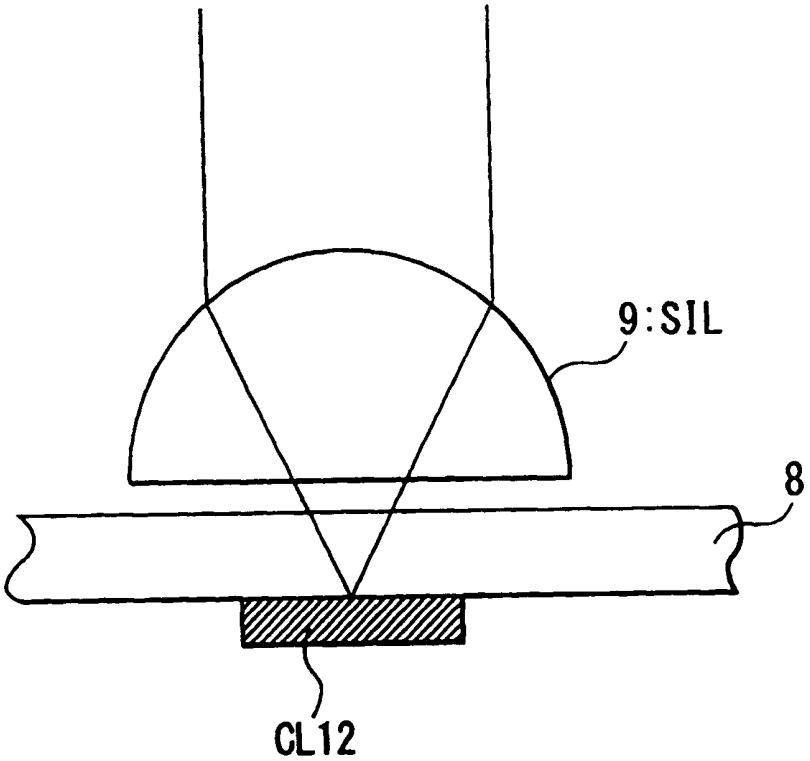
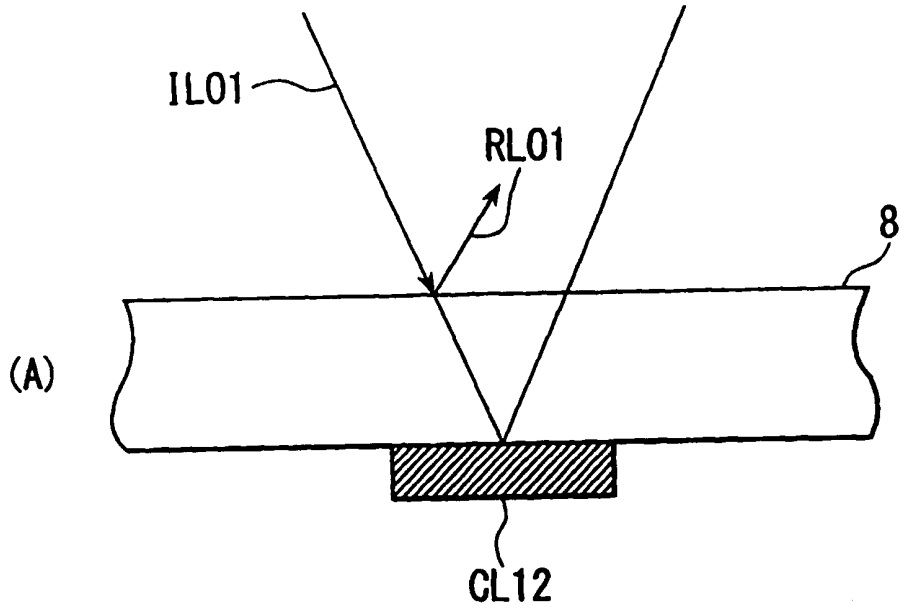


FIG. 19



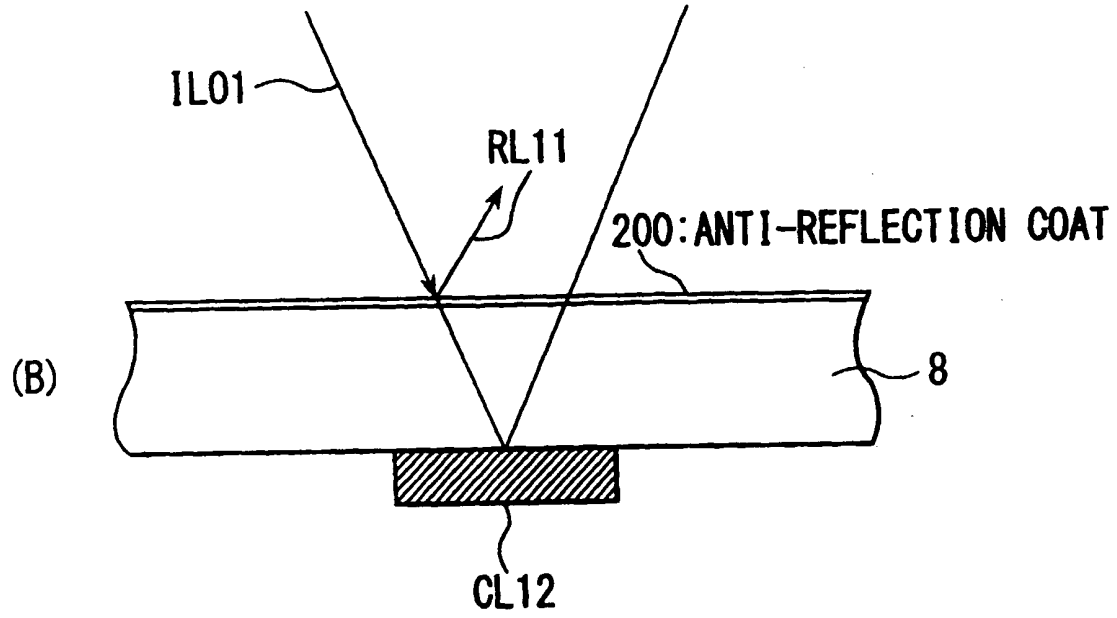


FIG. 20

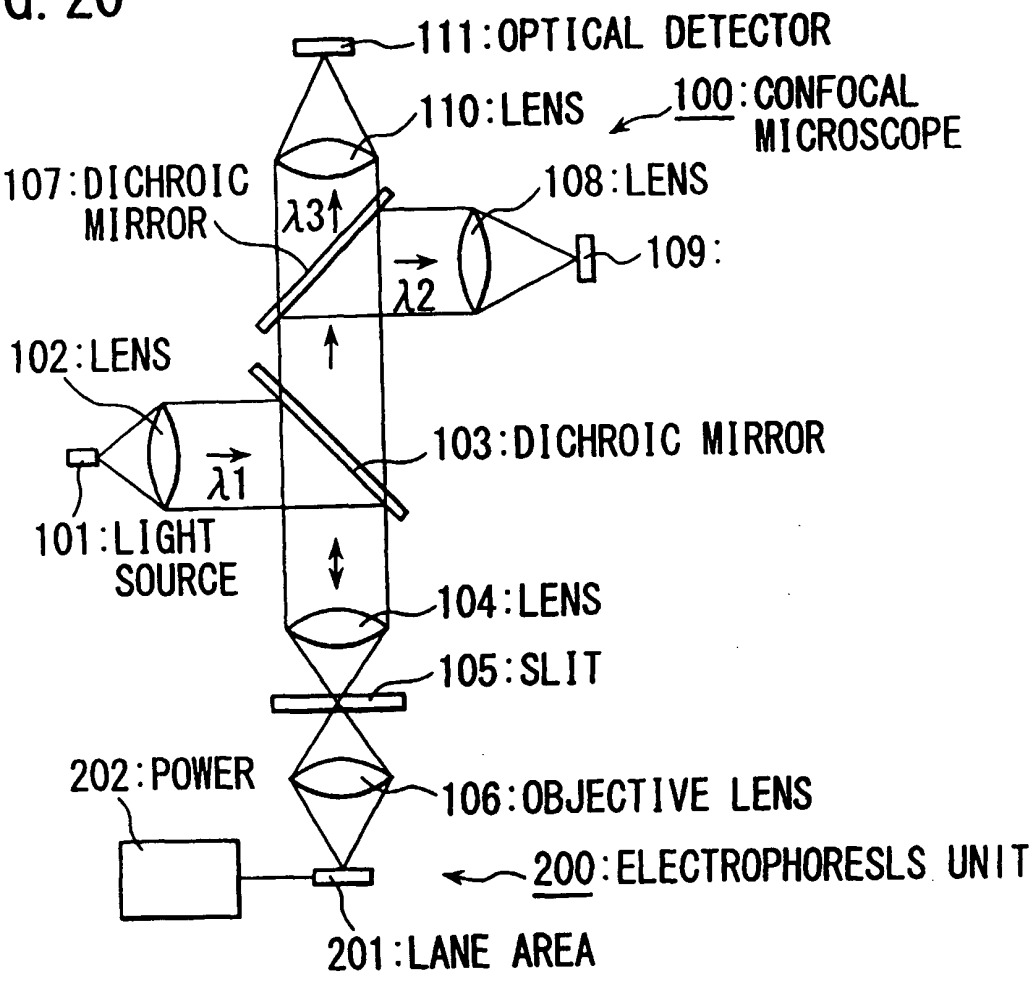

111:OPTICAL DETECTOR
110:LENS
100:CONFOCAL MICROSCOPE
107:DICHROIC MIRROR
λ3
108:LENS
109:
λ2
102:LENS
103:DICHROIC MIRROR
λ1
101:LIGHT SOURCE
104:LENS
105:SLIT
202:POWER
106:OBJECTIVE LENS
200:ELECTROPHORESLS UNIT
201:LANE AREA

FIG. 21

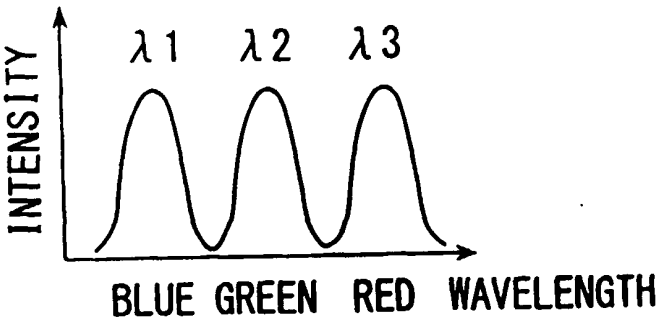

λ1
λ2
λ3
INTENSITY
BLUE GREEN RED WAVELENGTH

FIG. 22

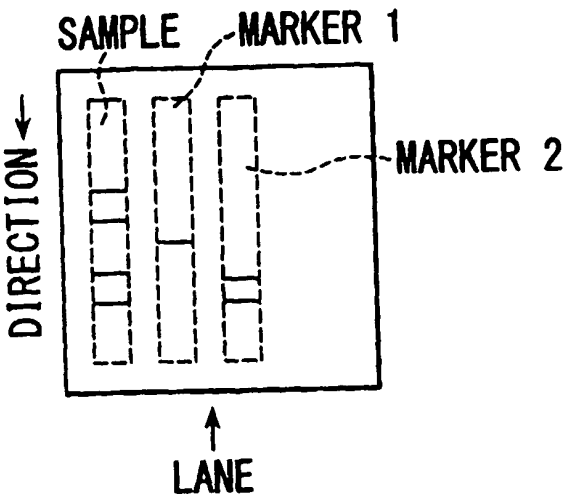


FIG. 23

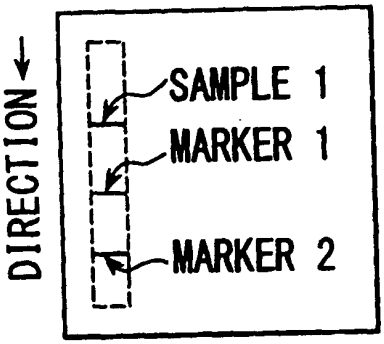


FIG. 24

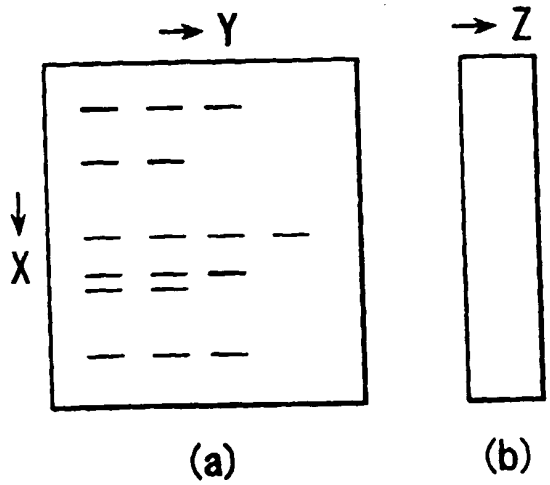


FIG. 25

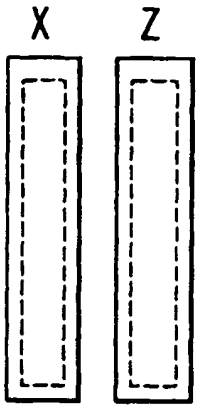


FIG. 26

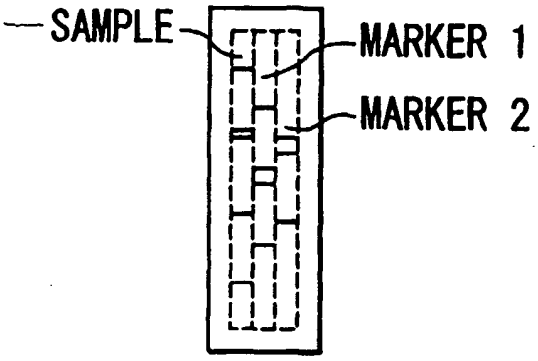


FIG. 27